Case HP/2-21551/A

#13
3-27-01

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF ANDREAS WERNER SUPERSAXO ET AL

Group Art Unit: 1619

SERIAL NO.: 09/306,006

Examiner: S. SHARAREH

FILED: JUNE 5, 1999

FOR: USE OF NANODISPERSIONS IN PHARMACEUTICAL END FORMULATIONS

Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION UNDER RULE 132

I, Andreas Werner Supersaxo, a citizen of the Swiss Confederation, residing in Baar, Switzerland, hereby declare:

1. That I am a co-inventor of the invention disclosed and claimed in the above identified patent application;

2. That I have been employed by Vesifact AG since January 1, 1998, specializing in research of nano-sized carrier systems for life science products;

3. That I am presently head of R & D, and have held this position since January 1, 1998;

4. That I am engaged in the research and development of nano-sized carrier systems for life science products;

5. That I consider myself an Expert in preparation of drug delivery systems, especially lipid based delivery systems such as liposomes, mixed micelles and microemulsions;

6. That prior to my employment at Vesifact AG, I was an employee of F.Hoffmann-La Roche AG Basel, Switzerland and of Syntex Research, Palo Alto, California, USA;

7. That I received my Ph. D. in pharmaceutics in 1986 at the Swiss Federal Institute of Technology, Department of Physical Pharmacy, Zurich, Switzerland;

8. That I am a named inventor in U.S. Patents Nos.: 5,376,646; 5,416,202; 5,470,582; 5,759,827 and 6,030,602.

9. That I carried out the following preparative Examples (1A), (1B), (2A), (2B), (3A) and (3B) with the resulting formulations (1a), (1b), (2a), (2b), (3a) and (3b):

Example (1A): Preparation of prephase (1a) according to example 5 of the present application:

(a) soybean lecithin, a phospholipid	9.00 g
(b) polysorbate 80, a polyethoxylated sorbitan fatty acid ester	34.00 g
(c) vitamin E acetate	36.60 g
(d) miglyol 812, the triglyceride of C_{10}-C_{12} fatty acids	13.00 g
(e) ethanol	7.40 g

Example (1B): Preparation of prephase (1b) according to US-A-5,997,888:

(a) soybean lecithin, a phospholipid	9.00 g
(b) polysorbate 80, a polyethoxylated sorbitan fatty acid ester	34.00 g
(c) vitamin E acetate	36.60 g
(e) ethanol	7.40 g

Example (2A): Preparation of prephase (2a), prepared according to example 6 of the present application:

(a) soybean lecithin, a phospholipid	17.30 g
(b) polysorbate 80, a polyethoxylated sorbitan fatty acid ester	34.00 g
(c) vitamin A palmitate (1.7×10^6 IU/g)	4.50 g
(d) miglyol 812, the triglyceride of C_{10}-C_{12} fatty acids	30.00 g
(e) ethanol	14.20 g

Example (2B): Preparation of prephase (2b) according to US-A-5,997,888:

(a) soybean lecithin, a phospholipid	17.30 g
(b) polysorbate 80, a polyethoxylated sorbitan fatty acid ester	34.00 g
(c) vitamin A palmitate (1.7×10^6 IU/g)	4.50 g
(e) ethanol	14.20 g

Example (3A): Preparation of prephase (3a), not exemplified in the present application:

(a) soybean lecithin, a phospholipid	11.30 g
(b) polysorbate 80, a polyethoxylated sorbitan fatty acid ester	34.00 g
(c) Coenzyme Q 10	5.00 g
(d) miglyol 812, the triglyceride of C_{10}-C_{12} fatty acids	45.20 g
(e) ethanol	4.80 g

Example (3B): Preparation of prephase (3b) according to US-A-5,997,888:

(a) soybean lecithin, a phospholipid	11.30 g
(b) polysorbate 80, a polyethoxylated sorbitan fatty acid ester	34.00 g
(c) Coenzyme Q 10	5.00 g
(e) ethanol	4.80 g

Method of preparation of formulations (1a), (2a) and (3a):

(a) Preparation of the prephases

The prephases were prepared according to the present application.

Briefly, component (a) was dissolved in component (e). To the resulting solution the components (d), (b) and (c) were added and mixed, if necessary with heating, until a homogeneous clear liquid was obtained.

(b) Preparation of the monodisperse dispersions

The corresponding monodisperse dispersions were prepared by adding the prephase obtained in step (a) to 10 mM phosphate puffer (pH 6) according to the present application.

Briefly, the phosphate puffer (e.g. 450 g) was placed, with stirring (e.g. magnetic agitator), at 50°C in a vessel. The liquid prephases obtained in step (a) were added to the phosphate buffer with stirring (e.g. magnetic agitator).

Monodisperse dispersions (1a), (2a) and (3a) were obtained.

Method of preparation of formulations (1b), (2b) and (3b):

(a) Preparation of the prephases

Component (a) was dissolved in component (e). To the resulting solution the components (b) and (c) were added and mixed, if necessary with heating, until a homogeneous clear liquid was obtained.

(b) Preparation of the polydisperse dispersions

The corresponding polydisperse dispersions were prepared by adding the prephase obtained in step (a) to 10 mM phosphate puffer (pH 6).

Briefly, the phosphate puffer (e.g. 450 g) was placed, with stirring (e.g. magnetic agitator), at 50°C in a vessel. The liquid prephases obtained in step (a) were added to the phosphate buffer with stirring (e.g. magnetic agitator).

Polydisperse dispersions (1b), (2b) and (3b) were obtained.

The determination of particle sizes and particle size distribution analysis of the monodisperse dispersions (1a), (2a) and (3a) and the polydisperse dispersions (1b), (2b) and (3b) were carried out using a photon correlation spectroscopy based particle sizer (Nicomp 380 Submicron Particle Sizer). Data reported in Table 1 correspond to intensity weighted particle size.

Results:

The results are listed in Table 1:

Table 1:

Formulation	Active ingredient	Triglyceride	Particle distribution	Particle diameter [nm]
(1a)	2. % b.w. vit E ac	Migylol 812	monodisperse	39.6 ± 13.7
(1b)	2. % b.w. vit E ac	- none -	polydisperse	1. population: 22.2 (9.9 %) 2. population: 78.9 (63.1 %) 3. population: 428.2 (27.0 %)
(2a)	0.45% b.w. vit A palm	Migylol 812	monodispers	22.6 ± 5.9
(2b)	0.45% b.w. vit A palm	- none -	polydisperse	1. population: 22.9 (1.9 %) 2. population: 171.0 (82.1 %) 3. population: 899.0 (15.9 %)
(3a)	0.5 % b.w. Q10	Migylol 812	monodisperse	49.6 ± 21.4
(3b)	0.5 % b.w. Q10	- none -	polydisperse	1. population: 11.3 (1.2 %) 2. population: 183.8 (29.6 %) 3. population: 886.1 (69.2 %)

10. I, Andreas Werner Supersaxo, further declare that the results in Table 1 surprisingly show that the triglyceride used in the formulations of the present invention have a very positive effect on the formation of nanodispersions. The formulations (1a), (2a) and (3a) comprising the triglyceride are monodisperse dispersions which can be regarded as nanodispersions and have a mean particle diameter of < 50 nm.

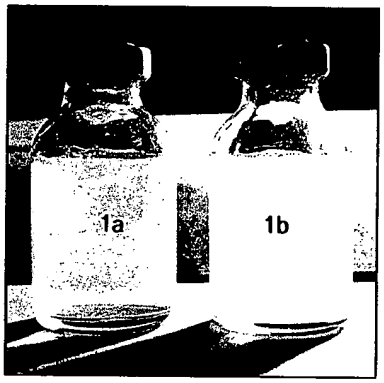
In contrast thereto the formulations (1b), (2b) and (3b) according to the teaching of US-A-5,997,888 are polydisperse dispersions, i.e. the dispersions contain two or more particle populations which differ in size. The mean particle diameters of these populations vary from 22.2 to 428.2 nm (vitamin E acetate), 22.9 to 899 nm (vitamin A palmitate) and 11.3 to 886.1 nm (coenzyme Q10) as can be seen from Table 1.

Polydisperse formulations are not suitable for pharmaceutical and/or cosmetic applications since the active ingredient is not present in a homogenous distribution in the formulation and can not be delivered in constant and well defined concentrations.

The differences of particle size and particle distribution can also be visualized as demonstrated in Table 2 wherein pictures of the formulations (1a) - (3b) were taken.

The results in the comparative test further show that nanodispersions of the present invention can be prepared very easily without additional supply of energy, i.e. without shear or cavitation forces.

Table 2: Visualization of the Difference in Particle Size and Particle Distribution



(1a) (1b)

(2a) (2b)

(3a) (3b)

12. I, Andreas Werner Supersaxo, further declare that all statements made herein of personal knowledge are true and all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed this 2nd day of March 2001.

Andreas Werner Supersaxo